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Pharmacokinetic and Pharmacodynamic Studies of Prednisolone Acetate

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ABSTRACT
Prednisolone acetate (PA) is a corticosteroid with potential application in the management of multiple sclerosis; however, effective delivery to brain tissue remains challenging. The present study evaluated an optimized prednisolone acetate solid lipid nanoparticle (PA-SLN) formulation for intranasal brain-targeted delivery and compared its pharmacodynamic and pharmacokinetic performance with conventional oral PA. The optimized formulation contained 11% tristearin, 58.88% surfactant mixture [Cremophor RH40:ethanol (3:1)] and a sonication time of 9.33 min. Male mice were divided into normal, diseased, oral PA and intranasal PA-SLN groups and treated for 21 days at 1.988 mg/kg in a cuprizone-induced multiple sclerosis model. Locomotor activity and motor coordination were assessed using actophotometer, open-field, rota-rod and swim tests, while brain histopathology evaluated demyelination and remyelination. PA concentrations in plasma and brain homogenate were quantified by HPLC. Intranasal PA-SLNs significantly improved locomotor activity and motor coordination and promoted faster remyelination compared with oral treatment.

INTRODUCTION

The optimized prednisolone acetate solid lipid nanoparticle (PA-SLN) formulation obtained from the preformulation and formulation studies demonstrated satisfactory physicochemical and formulation characteristics. The in vivo investigation incorporated pharmacodynamic assessments, including locomotor activity and motor coordination, to evaluate functional changes associated with the experimental disease condition and treatment. Histopathological examination of brain tissue was additionally performed to assess structural alterations and the extent of demyelination or remyelination. Drug concentrations in plasma and brain tissue were determined at predetermined time intervals following administration. Prednisolone acetate was quantified using a validated HPLC method, enabling assessment of drug exposure in both systemic circulation and brain tissue [1-4]. Pharmacokinetic evaluation was performed using plasma and brain concentration–time profiles to determine important parameters including Cmax, Tmax, elimination rate constant, half-life, AUC, AUMC, and mean residence time (MRT). The study also assessed drug-targeting parameters to determine the efficiency of intranasal delivery in achieving enhanced drug localization in the brain. Thus, the in vivo studies provided an integrated assessment of the therapeutic performance and brain-targeting potential of the optimized PA-SLN formulation in comparison with conventional oral prednisolone acetate therapy[5-6].

MATERIALS AND METHODS

Materials:

Male mice procured from Animal suppliers- Hyderabad. The in vivo work was conducted as per the procedure specified by institutional animal ethical committee, AMRMCP. Animals were placed under set environmental conditions i.e. 22 ± 2°C temperature and 12 hrs light/dark cycles. The animals were open access to normal diet (VRK Nutrition solution, Bangalore.) and water.

Methods:

The findings obtained after completion of preformulation and formulation studies have shown good results based on the experimental values of optimized formula comprising tristearin 11%, surfactant combination (cremophor RH40: ethanol (3:1) 58.88% and sonication time 9.33 min. Therefore, this refined formulation was taken up for additional in-vivo testing [7-8].

Study Protocol:

The male mice were randomly picked and divided into different groups as described in the Table 1 below. Animals of two distinct groups were given with needed amount of PA i.e. 1.988 mg/kg body weight for a period of 21 days thru oral and intra nasal route in animals. The animals were separated into four groups; Group-I & II comprised of 12 animals and Group-III & IV consisted of 48 animals. The animals were allowed to acclimate to the laboratory environment for one week before the experiment began. Animals were treated simultaneously for 21 days with four different preparations namely, Water for normal group (Group–I), cuprizone (0.4%) for diseased group (Group–II), cuprizone and prednisolone acetate tablet (orapred) for oral group (Treatment Group–III) dose: 1.988 mg/kg, cuprizone and drug embedded in solid lipid nanoparticles for Nasal group (Targeted preparation with dose of 1.988 mg/kg (Treatment Group–IV). The complete study procedure is below.

Table 1: Study Protocol

Study title	A study on effect of PA nanoparticles and tablet mediated drug delivery in the treatment of multiple sclerosis induced by cuprizone.
Study objective	To determine the rate and extent of nasal absorption of Prednisolone acetate compared with that of rate and extent of Prednisolone acetate oral absorption in multiple sclerosis.
Sample size	60 healthy black mice weighing 25 ± 1.2 g (8-weeks)
Drug	Prednisolone acetate solid lipid Nanoparticulate and tablet form
Dose	1.988 mg/kg
Study treatments	Group – I: under normal diet, Number of animals 6. Group – II: Cuprizone treatment, Number of animals 6. Treatment Group – III: Oral group- Cuprizone (toxicant) 0.4% + Prednisolone acetate tablet (orapred) at a dose of 1.988 mg/kg, number of animals 24. Treatment Group – IV: Nasal group- Cuprizone (toxicant) 0.4% + Prednisolone acetate embedded nanoparticles at a dose of 1.988mg/kg, number of animals 24.
Sampling schedules	Blood samples withdrawn from retroorbital area and brain samples were also collected at predetermined intervals such as 0, 0.5, 1, 2, 4,8, 12 and 16 hrs.
Bioanalytical method	Prednisolone acetate was estimated in brain and blood samples using HPLC technique.
Pharmacokinetics	Cmax, Tmax, AUC0-t, AUC0-∞, AUMC0-t, AUMC0-∞, kel, t1/2 and MRT were analyzed from the plasma concentration-time and brain homogenate-time data of prednisolone acetate using kinetica 5 software.
Ethical consideration	The study was carried out according to the norms of the CPCSEA

At the end of 21st day, after treatment with specified preparations all the animals are subjected to pharmacodynamic studies to confirm variation in different groups later a part of brains subjected to histopathological studies for confirmation of development of multiple sclerosis and the other part of brain subjected for quantification of drug in brains of the animals and quantification of drug in blood was also analyzed, followed by pharmacokinetic analysis.

Pharmacodynamic Studies [9-10]

Locomotor activity: Locomotor activity was examined using open field equipment and actophotometer.

Actophotometer: Male mice with a body weight of around 25 ± 1.2 g were divided into 4 groups and the animals were placed individually in a metal chamber 30 × 30 cm in size with a panel bottom and a firm cover. All the animals were scored for 5 min for base activity.

Open field test: The test equipment consists of a square space 76 × 76 with a wall height of 42 cm. The floor of the test apparatus was divided into 25 equal squares. For mobility, each animal was maintained at one end of an open field apparatus and the number of squares crossed was counted in 3 min.

Rotarod test:

The test equipment consisted of a rod coupled with a 7.0cm diameter drum. The speed of the rod was regulated to 12 revolutions/min during the testing operation. Motor coordination was assessed by the falling time of an animal throughout test period of 180s.

Despire swim test: Each animal was placed into a pool of water, maintained at 10 cm deep (45cm long; 22 cm broad circumference and 20 cm high). The animals were allowed to explore the forced swim rotations. The number of rotations/3 min was measured.

Histopathological studies

Histopathology and scoring of cuprizone induced demyelination Processing of isolated brain Brains were dissected following sedation using chloroform. The excess tissues of brain were removed and fixed in 4% paraformaldehyde. The brains were cleaned for around 10 min under running water and dehydrated by using different percentages of isopropyl alcohol such as 70%, 80%, 90% for 12 hrs and the last phase of dehydration was done using absolute alcohol. For the clearing of the tissue of brains, xylene was used for around 20 min. The brain tissues were cleaned and inserted in square shaped molds, and infiltrated with paraffin wax utilizing self-tissue embedding equipment.

Sectioning

The solidified tissue in the square wax blocks was sliced into thin pieces with microtome and had the thickness of 5 µm. The thin slices were put on a tiny glass slide pre-coated with gelatin. Then thin parts placed into hot air oven and held at 60°C till 1hr; At this temperature paraffin and gelatin were denaturated, thus tissue fixed correctly on the tiny glass slide.

Staining

The glass slide so made was rehydrated in 95% alcohol and kept overnight at 60°C in 0.01% Luxol fast blue solution (staining agent). The slices were differentiated in a 0.05% lithium carbonate solution and counterstained with cresyl violet.

In vivo study

Blood sampling

Blood samples were collected at different time intervals such 0, 0.5, 1, 2, 4, 8, 12 and 16 hrs from retroorbital region into EDTA tubes and was centrifuged using Remi centrifuge for plasma separation at 2500-3500 rpm for 15 min. The samples were used for measurement of prednisolone acetate using established HPLC technique.

Brain sampling

Samples from brain were obtained at various time intervals such as 0, 0.5, 1, 2, 4, 8, 12 and 16 hrs and homogenized using tissue homogenizer in ice cold lysis buffer pH 7.4. The homogenate was centrifuged by micro centrifuge at 10,000 rpm for 30 min and kept at refrigerated temperature for further analysis.

Analytical method and instrumentation

For the analysis of in vivo experiments, Agilent-HPLC system comprising of Agilent C18 column (250 mm x 4.6 mm, 5 µm particle size), UV-PDA detector and 25 µL Hamilton syringe was used.

Chromatographic conditions

The drug was resolved on an Agilent C18 column (250 mm × 4.6 mm, 5 µm particle size). Mobile phase composition was IPA: n-Hexane (5:95% v/v). The flow rate of the mobile phase was 2 mL/min and the eluate was monitored with UV detector at 250 nm.

Extraction procedure of prednisolone acetate from mice plasma: The plasma samples were spiked with 500 µL serum, 80 µl methanol and 100 µL dexamethasone methanolic solution (internal standard). Then 2 mL of ethyl acetate was added to this mixture and agitated for about 10 min then ultracentrifuged at

2500g for 10 min. Then organic layer was separated and placed in to glass cylinder and heated under nitrogen stream at 45oC. The residue was reconstituted in ethyl acetate (100 µl). 20 µL of the sample was introduced into the HPLC.

Extraction procedure of prednisolone acetate from brain homogenate: The separated brain was homogenized in an ice bath with a tissue homogenizer using a physiological buffer pH 7.4. Deproteinization was performed with 20% tricholoroacetic acid and 100 µL of internal standard solution was added to homogenate. The mixture was centrifuged at 1500g for about 10 min. The final supernatant was extracted for drug with ethyl acetate as described in plasma.

Pharmacokinetic analysis

The plasma concentration-time and brain homogenate concentration – time graph was plotted and several kinetic parameters including Cmax, tmax, Kel, t1/2, AUC0-t, AUC0-∞, AUMCo-t, AUMC0-∞ were measured using pharmacokinetic software (kinetica 5). The plasma profiles were utilized to calculate the Cmax and tmax after oral and IN administration. AUC was calculated using the trapezoidal technique. The drug targeting efficiency and the percentage of drug transport were determined. Table 6.7 and Fig. 6.12 indicate the concentration of drug in plasma and brain at different time intervals. Kinetic Parameters after Oral and IN Administration. Table 6.8.

RESULTS AND DISCUSSION

Pharmacodynamic studies

Table 2: Effect of PA formulations on locomotor activity (Actophotometer)

S.NO.	Group	Locomotor activity (No. of counts/5min)
1	Normal group	269.3±1.5
2	Diseased group	60.33±9.39
3	Nasal group	210.3±6.6**###
4	Oral group	151.7±9.10**##

**P < 0.01, when compared with Normal group and ##P <0.01, ###P<0.001, when compared with Diseased group.

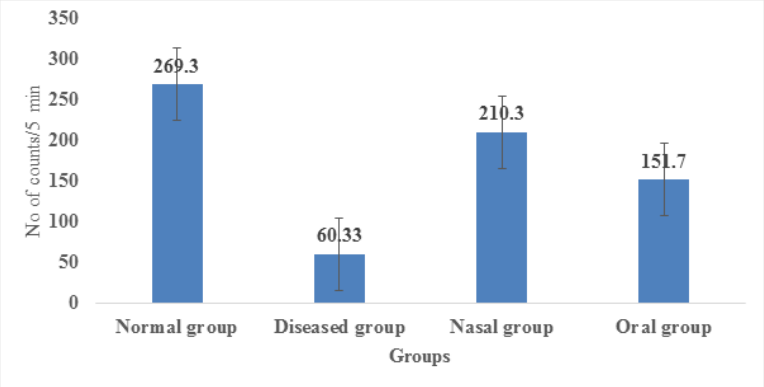

Fig 1: Effect of PA formulations on locomotor activity of mice (Actophotometer)

Locomotor activity was significantly decreased in the diseased group (60.33±9.39) as compared to the normal group (269.3±1.5). The nose group exhibited a significant increase in locomotor activity (210.3 ± 6.6) suggesting a stronger recovery than the oral group (151.7 ± 9.10). The considerable increase in the nasal group indicates that intranasal PA-SLN therapy has a better therapeutic effect than oral delivery.

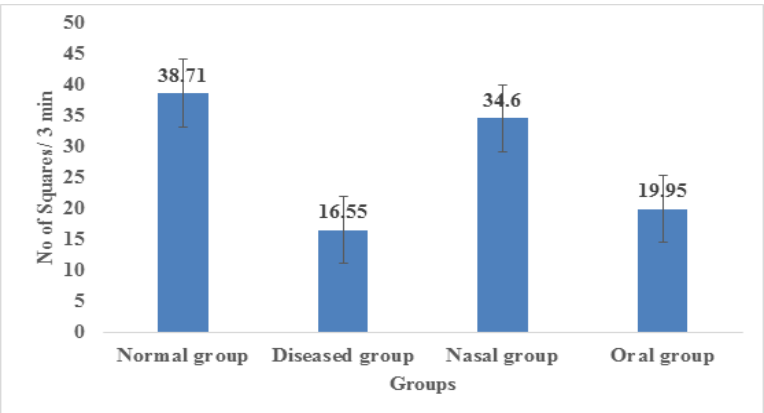

Fig 2: Effect of PA formulations on locomotor activity of mice (Open field)

In the open-field test, locomotor activity of the disease group (16.55 ± 0.9) was decreased in comparison with the normal group (38.71 ± 2.5). The nasal group showed remarkable improvement (34.6 ± 0.5), nearing the usual level of activity indicating significant recovery. The oral group exhibited only mild improvement (19.95 ± 1.1) which indicates superior effectiveness of intranasal formulation.

Table 3: Effect of PA formulations on locomotor activity (Open field)

S.NO.	Group	Locomotor activity (No. of Squares/3min)
1	Normal group	38.71 ± 2.5
2	Diseased group	16.55 ± 0.9
3	Nasal group	34.6 ± 0.5**##
4	Oral group	19.95 ± 1.1

**P < 0.01, when compared with Normal group and ##P<0.01, when compared with Diseased group.

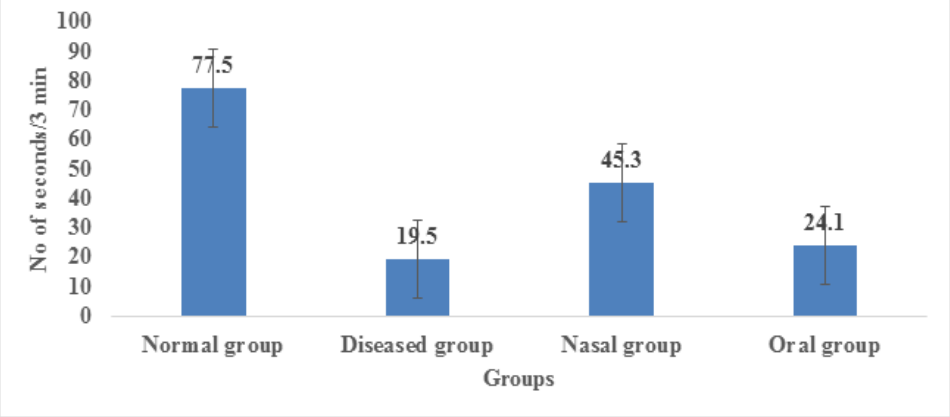

Fig 3: Effect of PA formulations on Motor coordination of mice (Rota rod)

The motor coordination of the diseased group was significantly lower (19.5 ± 3.1) than that of the normal group (77.5 ± 2.6). The nasal group had significantly higher improvement (45.3 ± 2.2) than the oral group (24.1 ± 2.2) with better motor recovery. Overall, the intranasal formulation showed a better improvement in motor coordination than the oral therapy.

Table 4: Effect of PA formulations on Motor coordination (Rota rod)

S.NO.	Group	Motor coordination (No. of Seconds/3min)
1	Normal group	77.5± 2.6
2	Diseased group	19.5 ± 3.1
3	Nasal group	45.3 ± 2.2***###
4	Oral group	24.1 ± 2.2**

**P < 0.01, ***P < 0.001, when compared with Normal group and ###P<0.001, when compared with Diseased group.

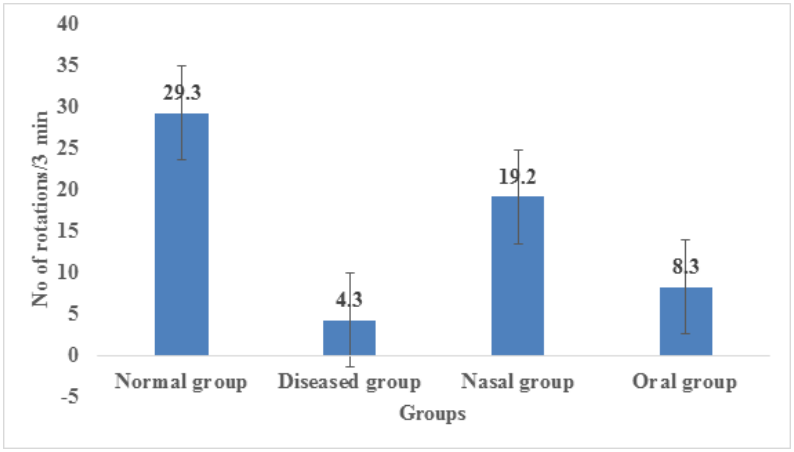

Fig 4: Effect of PA formulations on Motor coordination of mice (Swim test)

The motor coordination of the disease group was significantly lower (19.5 ± 3.1) than that of the normal group (77.5 ± 2.6). The nasal group had significantly higher improvement (45.3 ± 2.2) than the oral group (24.1 ± 2.2) with better motor recovery. Overall, the intranasal formulation showed a better improvement in motor coordination than the oral therapy.

Table 5: Effect of PA formulations on Motor coordination (Swim test)

S.NO.	Group	Motor coordination (No. of rotations/3min)
1	Normal group	29.3 ±0.65
2	Diseased group	4.3±0.84
3	Nasal group	19.2±3.1* ##
4	Oral group	8.3±0.33

*P < 0.05, when compared with Normal group and ##P<0.01, when compared with Diseased group.

Histopathological studies of brain tissue of mice brain

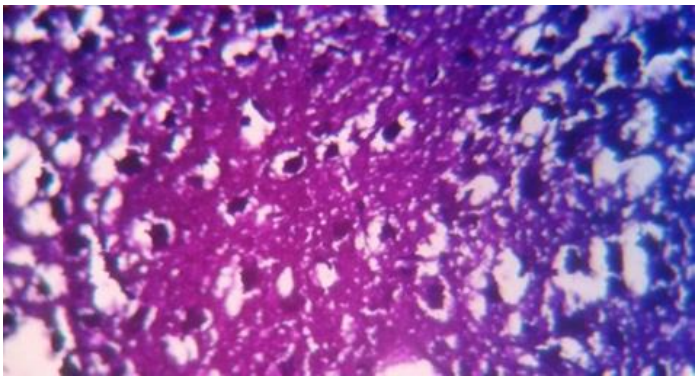

Fig 5: Normal tissue – Showing normal arrangement of myelin in regular fashion

Normal brain tissue reveals a regular and homogeneous distribution of myelin, with homogeneous staining and preservation of structural integrity, consistent with normal myelination without evidence of demyelination

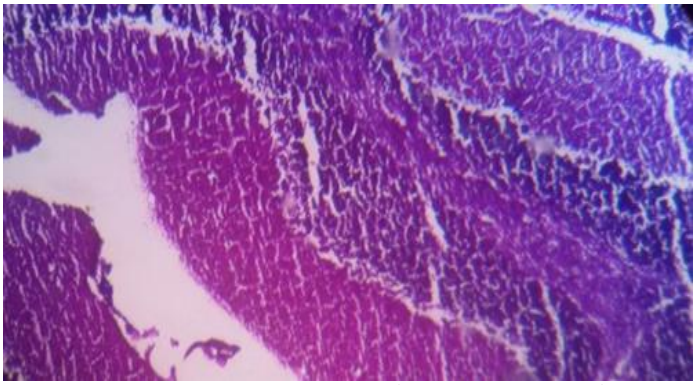

Fig. 6: Diseased control – Showing loss of structural integrity of myelin tissue

The diseased brain tissue demonstrated disruption and loss of normal myelin architecture with uneven staining and regions of myelin degradation, and exhibited severe demyelination and reduced structural integrity compared with normal tissue.

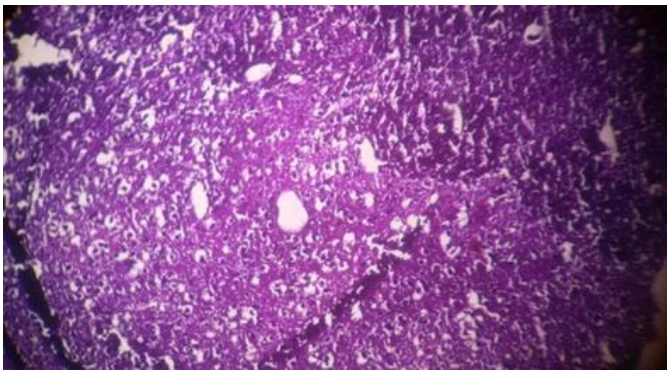

Fig.7: Intranasal treatment- Showing faster remyelination

The intranasal treatment group shows evidence of improved myelin organization with increased remyelination, reflected by restoration of myelin staining and improved tissue architecture compared with the diseased control. This indicates a faster regenerative response following intranasal PA-SLN treatment.

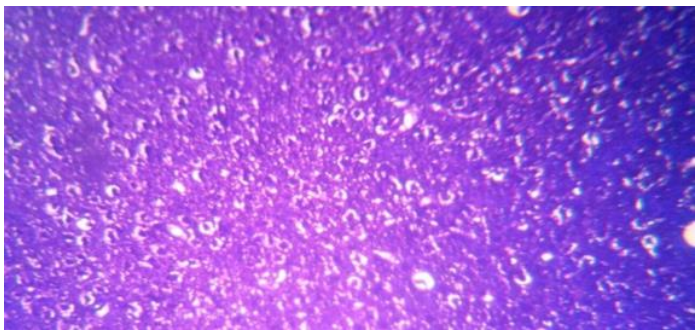

Fig. 8: Oral treatment- Showing slow remyelination

The oral treatment group shows partial restoration of myelin architecture with comparatively limited remyelination. The persistence of irregular myelin organization suggests a slower regenerative response than the intranasal PA-SLN treatment, indicating comparatively lower efficacy in promoting remyelination.

Standard calibration curve

Table 6: Prednisolone acetate standard calibration curve

Concentration (µg/mL)	Peak area
0	0
5	436415
10	853614
20	1758348
50	4680361
100	9275316

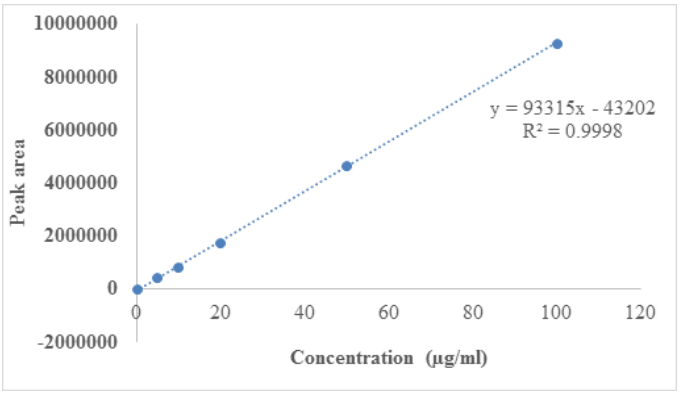

Fig. 9: Standard calibration curve of prednisolone acetate

The correlation coefficient (R2) was found to be 0.9998. The calibration curve values were shown in Table 6.6 and graph shown in Fig. 6.9. The linearity was found in within the range of 5-100 µg/ml of prednisolone acetate by adding a known concentration of dexamethasone was used as internal standard.

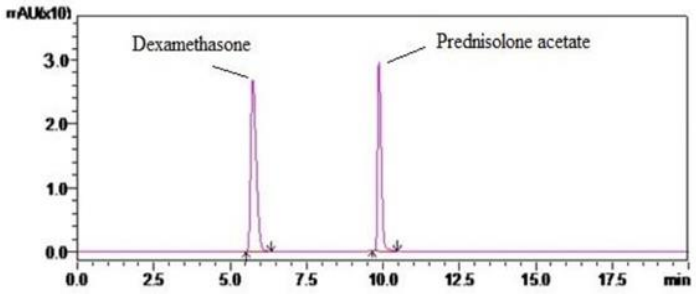

Fig.10: HPLC chromatogram of the controlled sample in plasma

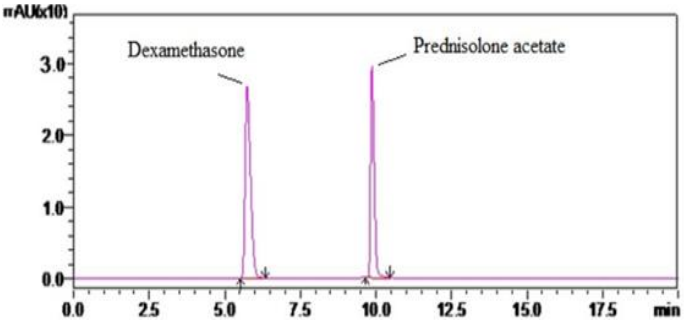

Fig 11: HPLC chromatogram of the controlled sample in brain

Homogenate

In the chromatograms of the controlled plasma and brain homogenate samples well-resolved peaks for the internal standard dexamethasone at around 5.8 min and prednisolone acetate at around 9.8 min are seen. The absence of considerable interference peaks around the analyte retention time confirms the good specificity and selectivity of the HPLC technique for prednisolone acetate in both biological matrices. The sharp, symmetrical peaks with a persistent baseline provide further proof of efficient chromatographic separation and accurate quantification of prednisolone acetate.

Pharmacokinetic assessment

Table 7: Plasma concentration profiles of prednisolone acetate at different time intervals

Time (hrs)	PA tablet - Oral (µg/mL)		PA SLNs - Intranasal (µg/mL)	
	Blood ± SD	Brain ± SD	Brain ± SD	Blood ± SD
0	0	0	0	0
0.5	2.2 ± 0.2	0.032 ± 0.25	2.11 ± 0.95	0.019 ± 0.012
1	4.1 ± 0.65	0.071 ± 0.01	3.49 ± 0.44	0.031 ± 0.002
2	6.1 ± 0.31	1.1 ± 0.12	6.19 ± 0.51	0.057 ± 0.003
4	7.9 ± 0.5	2.2 ± 0.51	9.27 ± 0.34	0.073 ± 0.001
8	10.99 ± 0.29	4.1 ± 0.3	11.14 ± 0.46	1.1 ± 0.31
12	4.95 ± 0.51	3.33 ± 0.41	12.28 ± 0.41	0.069 ± 0.002
16	2.1 ± 0.41	1.85 ± 0.13	8.1 ± 0.31	0.011 ± 0.002

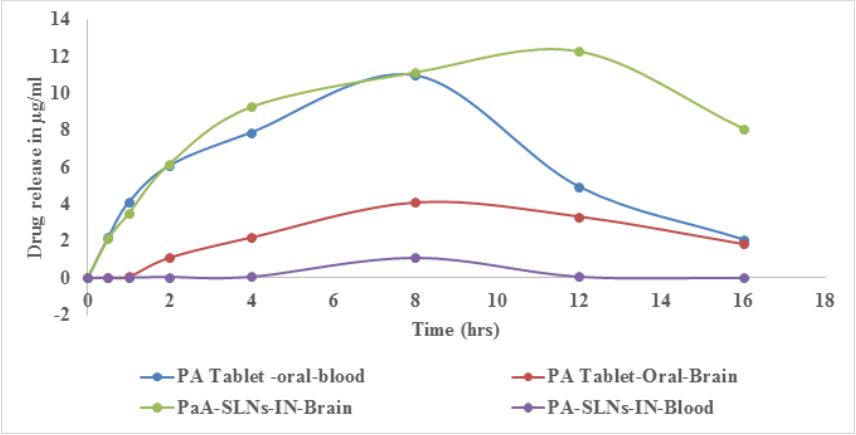

Fig. 12: Comparative plasma profiles of PA after oral and intranasal administration

Table 8: Pharmacokinetic data of prednisolone acetate

Parameters	PA tablet - Oral (µg/mL)		PA SLNs - Intranasal (µg/mL)	
	Blood ± SD	Brain ± SD	Brain ± SD	Blood ± SD
C_{max}(µg/mL)	10.99 ± 0.29	4.1 ± 0.3	12.28 ± 0.41	1.1 ± 0.31
T_{max} (h)	8 ± 0.01	8 ± 0.05	12 ± 0.06	8 ± 0.03
K_e (h^{-1})	0.158 ± 0.026	0.021 ± 0.047	0.0768 ± 0.117	0.0125 ± 0.096
$t_{1/2}$ (min)	263.16 ± 0.537	1980 ± 0.792	541.40 ± 0.628	3326.4 ± 0.481
AUC_{0-t} (µg.hr /mL)	84.14 ± 3.58	46.14 ± 1.74	157.85 ± 2.35	5.532 ± 9.82
$AUC_{0-\infty}$ (µg.hr/mL)	85.33 ± 0.98	137.57 ± 2.56	265.40 ± 6.28	11.05 ± 5.91
$AUMC_{0-t}$ (µg.hr^2 /mL)	485.26 ± 0.32	420.06 ± 0.94	1416.59 ± 0.85	43.75 ± 0.47
$AUMC_{0-\infty}$ (µg.hr^2 /mL)	489.49 ± 0.57	6236.66 ± 0.48	4537.84 ± 0.72	573.67 ± 0.26

The concentration-time profile showed a significant difference between oral PA and intranasal PA-SLNs. Prednisolone acetate showed a greater brain Cmax of 12.28 ± 0.41 µg/mL at 12h after intranasal treatment compared to 4.1 ± 0.30 µg/mL at 8 h after oral administration. The intranasal formulation also demonstrated significantly greater brain exposure, with enhanced AUC0–t (157.85 ± 2.35 µg•h/mL) and AUC0–∞ (265.40 ± 6.28 µg•h/mL), compared with oral PA (46.14 ± 1.74 and 137.57 ± 2.56 µg•h/mL, respectively). The much greater brain concentrations compared to the relatively low blood concentrations after intranasal injection suggest increased delivery of PA-SLNs to brain tissue with lower systemic exposure, indicating the promise of the intranasal route for brain focused delivery.

CONCLUSION

The in vivo investigations confirmed the therapeutic efficacy of the improved prednisolone acetate solid lipid nanoparticle (PA-SLN) formulation for brain-targeted distribution in the cuprizone-induced model of multiple sclerosis. The optimal formulation comprising 11% Tristearin, 58.88% surfactant combination (Cremophor RH40 (3:1)) and sonication period of 9.33 min was selected for in vivo assessment based on excellent formulation features. The pharmacodynamic assessment indicated that the intranasal PA-SLN formulation exhibited significant enhancement in locomotor activity and motor coordination as compared to diseased group and also showed enhanced functional recovery as compared to traditional oral formulation. The actophotometer, open-field, rota-rod and swim tests together suggested the better neurological function after intranasal injection. Histopathological analysis further confirmed the pharmacodynamic data. The disease animals showed loss of structural integrity of myelin and the intranasal treatment group showed quicker remyelination as compared to the oral treatment group demonstrating a positive impact of the targeted formulation on cuprizone induced demyelination.

CONFLICT OF INTERESTS: The authors declare no conflict of interest

ETHICS APPROVAL: Not applicable

FUNDING: This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY: Data will be available on request.

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